# Time of Mating and Incidence of Conception and Implantation in the Post Partum Lactating Female Mouse

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## Abstract of Thesis

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This study was conducted to investigate the time and incidence of post partum mating in the lactating mouse. The laboratory mouse exemplifies a phenomenon known as facultative (13) delayed implantation in which the post partum conception results in a prolongation of the normal 20 day gestation if the female is lactating. The lengthened gestation is dependent upon the number of suckling young (7,20), and the delay in implantation of the blastocyst results in the extended gestation (39).

Mating may be observed between the lactating female and male 12-24 hours post partum. The litter was sacrificed 24 hours post partum by etherization to investigate the possibility of the initial suckling stimulus causing the delay in implantation of the blastocyst. Since implantation sites could be observed in the uterus on day-5 of pregnancy, it

was concluded that the initial suckling stimulus is not entirely responsible for delayed implantation. The rates of conception and implantation were 40% and 29% respectively; but, there was no significant difference between the incidence of conception and implantation using a 95% confidence interval.

It is possible to increase the population of a mouse colony mating a lactating female mouse 12-24 hours post partum. Post partum mating would yield another litter before the conventional random mating method, because random mating depends upon the cyclic five-day estrus which does not begin until the twenty-second day of the lactation pregnancy.

Our former hypothesis was to use the pseudopregnant lactating female mouse as a recipient for ovum transfer; however, none of the transferred ova implanted. Neither using the lactating female as an ovum transfer recipient nor as a method to increase the size of a mouse colony seem feasible.

Accepted by: James R Dixon, Chairman
Margaret B Herald
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# Introduction

The laboratory mouse exemplifies a phenomenon known as facultative delayed implantation in which the post partum conception results in a prolongation of the normal 20 day gestation if the female is lactating. The lengthened gestation is dependent upon the number of suckling young (7,20), and the delay in implantation of the blastocyst results in the extended gestation (39).

The mouse has an excentric type of implantation in which the blastocyst becomes embedded in a depression of the endometrium as the uterine lumen closes over it (7). Prior to implantation, the blastocyst has no organic attachment with the uterine lumen and is isolated by the more or less attentuated zona pellicuda (7).

The intensity of mammary activity is proportional to the litter size up to seven or eight but shows no increased activity with larger litters (7).

Parturition, the terminal phase of embryonic life, lasts a few minutes in rodents and the onset of another pregnancy occurring within hours poses a most difficult problem in determining how lactation inhibits

implantation. Post partum mating usually occurs within the first 24 hours after the litter is born (4,9); but, post partum copulation can not be verified by the presence of a vaginal plug (9) -- as it is in non-lactating rodents.

Half-hour mating intervals were scheduled the first 24 hours post partum between the lactating female mouse and male to observe copulation. Assuming post partum mating could be observed, this study was conducted to investigate the incidence of conception and implantation in the post partum lactating female mouse. The oviducts were flushed 48 hours after mating to check for fertilized ova and implantation sites were examined in different individuals.

## Review of Literature

Copulation and conception occurs within 24 hours post partum (23), ovulation occurs between 12 and 32 hours post partum (29), and estrus occurs 12 to 18 hours post partum (1). Although copulation occurs, the incidence of pregnancy is decreased (19); however, if pregnancy does not result from this mating, ovulation next occurs 22 days post partum in most mice as proven by the presence of tubal ova (17). Inhibiting estrus by lactation in the mouse resembles delayed implantation of blastocyst during lactation (7).

Embryonic diapause can also be observed in plant seeds or bird eggs as there may be some delay before germination or incubation (26). Unlike plants or birds, the delayed development of mouse embryos seems to be hormonally regulated (26). ". . . The hormonally conditioned ovum growth-promoting factor of the uterus . . . " (35) acted in abeyance during delay in implantation. Cleavage of the ova occurs at a normal rate until the blastocyst stage where cleavage stops and the vesicle remains unattached in the uterus until implantation (20). Hormones regulate ovulation, fertilization, tubal transport of the ova, implantation,

and parturition in a given time sequence characteristic for a given species (39).

Post partum ovulating follicles measured 670u, about 350u during the next 11 days of lactation, and after the twelfth day, 400-450u which is comparable to the follicles two days prior to the post partum ovulation (8). The vagina contains more mucin during the latter half of pregnancy than during the first 11 days, and this higher degree of mucification correlates with the growth rate of the follicles and the corpora lutea of lactation (17). At parturition, the vagina appears highly mucified overlying a multilayered epithelium similar to the vagina in late pregnancy (17).

In both suckling and non-suckling females (23), the ova were in the two-cell stage during the first day of gestation, morula during the fourth day, and blastocysts during the fifth day of gestation (cited by Brambell 1937). Bindon (4) stated there was a significant difference between the number of zygotes recovered from normal and lactating females on the fourth day of pregnancy.

The blastocyst does not undergo further development after reaching the uterus until implantation

(7,26). The resumption of growth in the blastocyst occurs with the same abruptness as delay began (39). The embryos surviving in the lactating female decreased 30% between ovulation and parturition (17).

Observing the zygote development revealed that the delayed implanting blastocyst was equivalent to the early day-5 zygote of normal pregnancy (4).

Implantational delay of post partum ovulated ova has been ascribed to the inhibitory influence of the mammary glands upon the uterus and the metabolic features of the individual (19). The blastocysts in the lactating rodent are inhibited by protein deficiency and other undetermined uterine factors (13). Delay of implantation is dependent on the suckling of the female for at least three days (4). Maximum mammary development in the mouse occurs the tenth day of gestation, but implantation rarely occurs before the sixteenth day (42). Young begin eating solid food about the fifteenth day of gestation (7).

Lactational delayed gestation prevents excessive lactation and extensive physiologic and metabolic demands on the female from litters too closely spaced (26).

Delayed implantation occurs consistently in mice who have had one to four litters; however, the incidence of delay is reduced after five or more litters. It was not known whether this was due to parity or age or both (4). An exception to delayed implantation occurs in the C3H strain which repeatedly delivered litters every 21 days despite the fact they were suckling young. Post partum mating occurs within 24 hours after birth and there was no delay in implantation in the C3H strain (26).

The embryo's nutritional requirements are derived from the ovum's reserve supplements and the contents of the fallopian tube and uterus. Nutrition is affected by the phagocytic and absorptive capacities of the trophoblast and the placenta (7). Dickmann (14) postulates the existence of a uterine factor that was detrimental to the rat blastocyst. Delayed implantation results from insufficient proteins or a specific protein necessary for active growth of the blastocysts (14).

Early indications were that the inhibitory effect on the blastocysts was exerted through the lack of nutrients rather than the effect on the uterine endometrium (7). Thus, lactation would affect the

rate of development by lessening the food supply ultimately slowing the rate of development (22), but this was proposed before Kirkham demonstrated the delay occurred before implantation (cited by Brambell 1937; Enders 1963; Hamlett 1935). Lactational influence upon the blastocyst seems improbable; since, the nutritional drain would have been only infinitesimal as compared to the entire physiologic and metabolic aspects of the parent organism (7).

When implantation occurs, the growth is resumed at a normal rate and the young are born at the normal stage of development (20). The prolonged gestation is due to the extended quiescent period of the blastocyst (20), and the arrest in the blastocyst's development is due to a delay in implantation either exerted by the uterine endometrium and/or by some undetermined factor involving the blastocyst (7).

Implantation is promptly induced in suckling mice by the removal of the litter during delay in implantation (31). The prolongation is proportional to the number of suckling young (39). Snyder stated that each suckling pup prolongs gestation about one day; however, this is not a rule even though the

greater the number in the litter, the later the implantation and consequently the longer the gestation (39). The critical litter size inducing delayed implantation is three to nine pups per female (4). On the average, physiologic lactation delay of implantation in the mouse is prolonged nine days (26). Animals suckling three or more young exhibit prolonged gestation from two to sixteen days (7). A straight regression line of the form $Y = 0.84 + 3.94$ , where Y = the prolongation in days and X = the number of young suckled, can be used when three or more pups are suckled (7).

If the number of suckling young following post partum mating exceeds two, the duration of pregnancy is prolonged when lactation and gestation occurs simultaneously (7). Implantation immediately follows termination of lactation (7).

Over 300 years ago, William Harvey first recognized delayed implantation in the roe deer (37) Capreolus capreolus (cited by Lanman 1970). Ziegler's article (45) on the biology of the roe deer was the earliest reference; yet, no embrylogical examination was made (cited by Hamlett 1935). Bischoff (6)

described the delayed implanting ovum of Capreolus (cited by Hamlett 1935). Although it is easier to understand why it would be undesirable for the roe deer to deliver in mid-winter, it is more difficult to understand why the roe deer does not mate in late fall like all other deer (26).

Lataste (28) concluded that the slowing down or cessation of development during the cleavage stages resulted in the delay in implantation in mice mated at the post partum estrus; hence, a prolongation of gestation (cited by Bindon 1969c; Enders 1963; Hamlett 1935; Snyder 1938).

Daniel reported (12) ten instances of gestation prolonged two to ten days in mice suckling three to ten young (cited by Brambell 1937). The length of gestation varied directly with the number of young suckled. Neither Daniel (12) nor King (22) saw delayed implanting blastocysts (cited by Enders 1963; Hamlett 1935). Kirkham (23) recovered free blastocysts from mice suckling young. This constitutes the fourth species for which actual embryological evidence of the quiescent stage is available (cited by Hamlett 1935).

The intensity of mammary activity is proportional to the litter size up to seven or eight but shows no increased activity with larger litters (7).

Kirkham (23) concluded copulation occurred within 24 hours of parturition when he sacrificed mice daily from six to twenty-four days post partum that were suckling three to eight young and compared their embryos with a standard series from non-suckling females which had become pregnant at the post partum estrus (cited by Brambell 1937).

Females suckling three to eight young were sacrificed six to fourteen days post partum, and their blastocysts were found free in the uterine lumen. In lactating females sacrificed fifteen to twenty-four days post partum, the blastocysts were implanted. Kirkham (23) concluded implantation occurred at the end of the thirteenth day of pregnancy (fourteenth day post partum) and thought the delayed implantation was due to the loss of surplus nourishment through the mammary glands preventing the uterine mucosa reacting to the blastocysts (cited by Brambell 1937).

Although there has been more interest in the mouse and rat since 1930, the information has not

added materially to the works of Lataste (27, cited by Brambell 1937; Enders 1963; 28, cited by Bindon 1969c; Enders 1963; Hamlett 1935; Snyder 1938) and Kirkham (23, cited by Brambell 1937; Hamlett 1935).

Determining how lactation inhibits implantation posed a most difficult problem for parturition represents the terminal phase of embryonic life and lasts a few minutes in rodents and the onset of another pregnancy occurs within hours (39).

Parkes and Bellerby (34) showed the lactational inhibitory effect on estrus was exerted via the ovaries presumably by the corpora lutea.

The corpus luteum of the mouse (33) is essential to maintain pregnancy during the whole gestation period because ablation promptly results in the interruption of pregnancy. Progesterone, secreted by the corpus luteum, is responsible for the sensitization of the uterine endometrium (7). The functioning of the corpora lutea is prolonged in mice suckling a succeeding foster litter (39).

Mirskaia and Crew (32) suggested delayed implantation was due to the inability of the corpus luteum to provide sufficient luteal hormone for the double demand

of maintenance of pregnancy and lactation (cited by Brambell 1937; Enders 1963). Since lactation proceeds with the ablation of the ovaries (11), Mirskaia and Crew's theory was discarded.

During lactation, estrus is inhibited by the progestational effect of the corpus luteum if a litter of more than two pups is maintained (7).

There are histological features suggesting progesterone is secreted in the lactating mouse until 20 days post partum (17). The stromal nuclei of the uterine endometrium are enlarged; follicular growth is retarded; ovulation is inhibited until 22 days post partum; the vagina is mucificed after 12 days post partum; and the corpora lutea of lactation increases morphologically. Even though there is ample morphological evidence for the secretion of progesterone during lactation in the mouse, the amounts are apparently insufficient to elicit a uterine mucosa response to the quiescent blastocyst (18).

Lactation in the mouse is divided into two periods: (i) before the eleventh day of pregnancy, progesterone and negligible amounts of estrogen are present (ii) this is followed by increasing levels of estrogen

and a gradual decrease of progesterone (17). The luteal hormone induces and sensitizes the progestational uterine changes and conditions for implantation to take place during lactation (7).

Significant loss of endogenous estrogen in the milk has not been proven (40) even though injected estrogen is secreted by this route. Since continuous lactation pregnancies reduces the effect of lactation on implantation (25), it is difficult to account for the delay in implantation as a loss of estrogen in this manner. Moreover, Parkes and Bellerby (34) showed that spayed lactating rodents responded to minimal doses of estrogen suggesting little estrogen was lost through lactation.

Decreased estrogenic activity could result from increased loss or utilization of estrogen, increased antagonism to the hormone, or a decreased secretion (42). Increased antagonism and increased loss seemed unlikely (25); because, one would expect these to occur in all pregnancies during lactation and not to be reduced by a continuous succession of lactation pregnancies (42). Histological features and decreased ovarian weight indicated decreased ovarian activity

and reduced estrogen secretion (42). Ovarian estrogen production increases about five hours after litter removal (5).

Implantation is interrupted when the pelvic nerves are severed before copulation (10,24). The neuro-hormonal centers in the pituitary gland appear to play key roles in the initiation of implantation during lactation pregnancy (2,3). The suppression of pituitary secretion is a neurally regulated response to the suckling stimulus (36). Lactational delay in the rodent provides an ideal model to study the role of the pituitary gland in implantation (4). The development of the ovarian follicles are regulated by the pituitary gland (38), and ovulation is dependent on the anterior hypophysis (16,41).

The induction of implantation promptly after the removal of the suckling stimulus suggests two mechanisms for the release of gonadotrophins. (i) Removal of the litter could result in gonadotrophin release which is initially stimulated by innervation of copulation, or (ii) a reduced secretion of prolactin may result in gonadotrophin release (30).

Reduced ovarian secretion depending upon

gonadotrophin may result from reduced pituitary secretion, loss of gonadotrophin in the milk, or a refractory state of the ovaries (42). Estrogen deficiency results from the reduced production of gonadotrophin associated with the secretion of luteotrophin by the pituitary (43). Inadequate synergism between progesterone and estrogen prolongs gestation in lactating mice by inhibiting the blastocysts (43).

Gonadotrophins initiating implantation are released immediately in lactating rodents after removing the litter(44).

Corpora lutea were maintained functional by sufficient prolactin secreted by the pituitary resulting from the neuro-hormonal stimulus of suckling (17). Progesterone inhibits the release of gonadotrophic hormones from the pituitary (17). During the first ten days of lactation, the ovarian follicles are small and the estrogen secretion is negligible (17).

The prolactin titer declines ten to fifteen days post partum (21). Progesterone declines sufficiently permitting additional gonadotrophins to be released (17). The increased gonadotrophins caused an

enlargement of the follicles and result in the accelerated secretion of estrogen (17). After removal of the litter or as the young begin eating solid food, suckling lessens and the secretion of prolactin drops lower and the lactational corpora lutea becomes non-functional (17). The lactational delay of implantation results, at least in part, because of the reduced secretion of gonadotrophins by the pituitary and the consequent decrease in estrogen production (42).

An increased release of follicle-stimulating hormone (FSH) is associated with the lactation pregnancy. The significant decrease of FSH two hours after litter removal is interpreted as a release of the hormone (5). The low pituitary FSH content at eight hours after litter removal indicates continued FSH release; however, the higher value at 32 hours could designate retention of pituitary FSH (5). This extended release of FSH seemed necessary for implantation in suckling mice, for Bindon (4) showed that removal of the litter for eight hours did not interrupt the delayed implantation. Gonadotrophin secretion is decreased during lactation (42), and increases before implantation in the lactating mouse (43).

## Materials and Methods

A random bred pigmented strain of female mice 8-10 weeks old were mated to pigmented males. Day-1 of pregnancy was the morning that a vaginal plug was observed. Two day before parturition, the females were caged individually so the female would not be influenced by other births in the same cage. After the litter was born, the female was placed in a cage with a male while her litter was nourished by a lactating foster female. The mother was reunited with her litter the following morning. Copulation could not be checked by observing a vaginal plug because the physiology of the vagina and uterus had not stabilized from the preceding pregnancy.

The foster female did not always accept the responsibility of lactating for the foster litter. Either she did not lactate and the litter died or she destroyed the litter physically.

Sometimes the mother would destroy her own litter when reunited with them or would not accept them and the litter died.

Exacting procedures must be employed to bring about successful mating of the post partum female. The

male was placed in the cage with the individual female before and at parturition, and both were unsatisfactory. The agression of male during this period combined with the regression of the female created turmoil; usually destroying the litter.

The next approach was to observe the male and female while they were together. The litter size was adjusted to eight per female immediately after parturition. Since lactational estrus occurs 12-18 hours post partum (1), the female was placed with a male after the litter was born. This time period was in half-hour intervals for the first 24 hours post partum. This eliminated the foster female and the presence of the male at parturition. During the first 12 hours post partum, the female resisted copulation; but, mating occurred a few minutes after the female was placed with a male during the next 12 hours.

Hence mating could be observed 12-24 hours post partum alleviating the tragic first 12 hours, and the incidence of mating could be calculated.

The litter was weaned 24 hours post partum to determine whether the initial suckling stimulus was responsible

for prolonging implantation to day-13 of lactation pregnancy. Laparotomy on day-5 of lactation pregnancy revealed implantation sites in the uterus indicating the initial suckling stimulus was not entirely responsible for prolonging implantation.

The incidence of conception was determined by flushing the oviducts of the female who had copulated during the post partum estrus and had been weaned 24 hours post partum. The oviducts were excised 48 hours after copulation. After sacrificing the mouse by cervical separation, a mid-abdominal incision exposed the reproductive system after the intestines were pulled aside. Holding the uterus with forceps, the bursa (which surrounds the fimbriated end of the oviduct adjacent the ovary) and the uterotubal junction were excised. A 30-gauge needle afixed to a 2.5 ml syringe was inserted into the fimbria of the oviduct and the contents were flushed with saline into a culture dish. This procedure was performed with the aid of a binocular stereoscope at 32X.

## Results

A litter size of eight per female was maintained from birth until the litter was weaned on day 20 of lactation pregnancy to investigate whether lactation would prolong gestation.

Two incidences of prolonged gestation were recorded during the preliminary observation (see Table 1). The delays in the mice tested were 14 and 11 days respectively.

Table 1. Prolonged gestation in lactating mice

| Mouse | First litter born | Post partum mating | First litter weaned | Second litter born |
|---|---|---|---|---|
| 1 | 11-04-72 | 11-05-72 | 11-25-72 | 12-08-72 |
| 2 | 11-05-72 | 11-06-72 | 11-26-72 | 12-06-72 |

Data regarding conception in a total sample of 30 mice are shown in Tables 2 and 3. The rate of conception was calculated by the following analysis.

$$f_e = \frac{\text{Number of successes}}{\text{Sample size}}$$

$$f_e = 12/30$$

$$f_e = 0.40$$

A 95% confidence interval was given to $P_e$ (the true percentage) when the upper and lower limits of conception were calculated. Let C = 0.05.

$$U = f_e + Z_{C/2}\sqrt{\frac{f_e(1 - f_e)}{n}}$$

$$U = 12/30 + 1.96\sqrt{\frac{(12/30)(18/30)}{30}}$$

$$U = 0.58$$

$$L = f_e - Z_{C/2}\sqrt{\frac{f_e(1 - f_e)}{n}}$$

$$L = 12/30 - 1.96\sqrt{\frac{(12/30)(18/30)}{30}}$$

$$L = 0.22$$

Table 2. Oviducts containing ova following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Flushed oviduct | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | 2-cell | 4-cell | 8-cell |
| 611 | 5-22 | 6-10 | @0900 | 12 | 6-11 | 1000 | 6-13 | 1300 | 8 | 6-13 | 1300 | + | + | |
| 612 | 5-22 | 6-10 | @1200 | 13 | 6-11 | 1000 | 6-13 | 1300 | 8 | 6-13 | 1300 | + | + | |
| 614 | 5-22 | 6-10 | @1100 | 14 | 6-11 | 1000 | 6-13 | 1200 | 7 | 6-13 | 1200 | + | + | + |
| 616 | 5-23 | 6-11 | 0900 | 11 | 6-12 | 1000 | 6-14 | 1000 | 8 | 6-14 | 1000 | | + | |
| 622 | 5-24 | 6-12 | 0900 | 12 | 6-13 | 1000 | 6-14 | 0900 | 8 | 6-15 | 1100 | + | + | + |
| 623 | 5-24 | 6-12 | 0900 | 11 | 6-13 | 1000 | 6-14 | 0900 | 8 | 6-15 | 1100 | + | | |

# This chart refers to the year, 1973.

(continued on page 23)

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition.

@ At that hour; otherwise, the birth had occurred.

Table 2. Oviducts containing ova following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Flushed oviduct | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | 2-cell | 4-cell | 8-cell |
| 628 | ? | 6-13 | @0900 | 12 | 6-14 | 0900 | 6-14 | 0900 | 8 | 6-16 | 1100 | | + | |
| 630 | ? | 6-13 | ? | 09 | 6-14 | 0900 | 6-14 | 1500 | 8 | 6-16 | 1100 | | + | |
| 631 | 5-26 | 6-15 | 0900 | 06 | 6-15 | 0900 | 6-16 | 1000 | 7 | 6-17 | 1100 | | + | |
| 634 | 5-26 | 6-14 | 0900 | 13 | 6-15 | 0900 | 6-15 | 0900 | 8 | 6-17 | 1100 | | + | |
| 635 | 5-26 | 6-15 | 0900 | 11 | 6-15 | 0900 | 6-16 | 1000 | 8 | 6-17 | 1100 | + | + | |
| 637 | 5-26 | 6-15 | 0900 | 06 | 6-15 | 0900 | 6-16 | 1000 | 8 | 6-17 | 1100 | + | + | |

# This chart refers to the year, 1973.

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition.

@ At that hour; otherwise, the birth had occurred.

Table 3. Oviducts without viable ova following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Flushed oviduct | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | Fragmented | Unfertilized | No ovulation |
| 608 | 5-22 | 6-10 | @1100 | 11 | 6-11 | 1000 | 6-13 | 1300 | 8 | 6-13 | 1300 | + | | |
| 613 | 5-22 | 6-10 | @1800 | 09 | 6-11 | 1000 | 6-13 | 1200 | 8 | 6-13 | 1200 | | | + |
| 615 | 5-22 | 6-10 | @1400 | 12 | 6-11 | 1000 | 6-13 | 1100 | 8 | 6-13 | 1100 | + | | |
| 617 | 5-23 | 6-11 | @1300 | 11 | 6-12 | 1000 | 6-14 | 1000 | 8 | 6-14 | 1000 | | + | |
| 620 | 5-23 | 6-11 | 0900 | 12 | 6-12 | 1000 | 6-14 | 1000 | 8 | 6-14 | 1000 | | | + |
| 624 | 5-24 | 6-12 | 0900 | 07 | 6-13 | 1000 | 6-14 | 0900 | 8 | 6-15 | 1100 | | | + |

# This chart refers to the year, 1973.

(continued on page 25)

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition.

@ At that hour; otherwise, the birth had occurred.

400100

Table 3. Oviducts without viable ova following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Flushed oviduct | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | Fragmented | Unfertilized | No ovulation |
| 625 | 5-25 | 6-13 | @0900 | 14 | 6-14 | 0900 | 6-14 | 0900 | 8 | 6-16 | 1100 | + | | |
| 627 | ? | 6-13 | @0900 | 11 | 6-14 | 0900 | 6-14 | 0900 | 8 | 6-16 | 1100 | + | + | |
| 629 | ? | 6-13 | 1300 | 06 | 6-14 | 0900 | 6-14 | 1100 | 8 | 6-16 | 1100 | + | | |
| 632 | 5-26 | 6-14 | @0900 | 09 | 6-15 | 0900 | 6-15 | 0900 | 7 | 6-17 | 1100 | + | | |
| 633 | 5-26 | 6-14 | @0900 | 10 | 6-15 | 0900 | 6-15 | 0900 | 8 | 6-17 | 1100 | | + | |
| 636 | 5-26 | 6-14 | 0900 | 12 | 6-15 | 0900 | 6-15 | 0900 | 8 | 6-17 | 1100 | | + | |

# This chart refers to the year, 1973.

(continued on page 26)

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition.

@ At that hour; otherwise, the birth had occurred.

Table 3. Oviducts without viable ova following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Flushed oviduct | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | Fragmented | Unfertilized | No ovulation |
| 639 | 5-26 | 6-14 | 0900 | 15 | 6-15 | 0900 | 6-15 | 0900 | 8 | 6-17 | 1100 | + | + | |
| 641 | 5-26 | 6-14 | 0900 | 12 | 6-15 | 0900 | 6-15 | 0900 | 8 | 6-17 | 1100 | + | + | |
| 642 | 5-27 | 6-15 | @1200 | 05 | 6-16 | 1000 | 6-16 | 1200 | 5 | 6-18 | 1000 | + | | |
| 643 | 5-27 | 6-15 | 0900 | 11 | 6-16 | 1000 | 6-16 | 0900 | 8 | 6-18 | 1000 | + | | |
| 644 | 5-27 | 6-15 | @0900 | 09 | 6-16 | 1000 | 6-16 | 0900 | 8 | 6-18 | 1000 | + | + | |
| 645 | 5-28 | 6-16 | 0900 | 12 | 6-17 | 1000 | 6-17 | 1000 | 8 | 6-18 | 1000 | | + | |

# This chart refers to the year, 1973.

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition (except 642).

@ At that hour; otherwise, the birth had occurred.

Data regarding implantation in a total sample of 55 mice are shown in Tables 4 and 5. The rate of implantation was calculated by the following analysis.

$$f_i = \frac{\text{Number of successes}}{\text{Sample size}}$$

$$f_i = 16/55$$

$$f_i = 0.29$$

A 95% confidence interval was given to $P_i$ (the true percentage) when the upper and lower limits of implantation were calculated. Let C = 0.05.

$$U = f_i + Z_{C/2}\sqrt{\frac{f_i(1 - f_i)}{n}}$$

$$U = 16/55 + 1.96\sqrt{\frac{(16/55)(39/55)}{55}}$$

$$U = 0.41$$

$$L = f_i - Z_{C/2}\sqrt{\frac{f_i(1 - f_i)}{n}}$$

$$L = 16/55 - 1.96\sqrt{\frac{(16/55)(39/55)}{55}}$$

$$L = 0.17$$

Table 4. Implantation sites observed following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Laparotomy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | Implanted | Not implanted |
| 508 | 5-09 | 5-28 | @2000 | 09 | 5-29 | 0900 | 5-29 | 2000 | 8 | 6-05 | 1100 | + | |
| 510 | 5-09 | 5-27 | 2200 | 14 | 5-28 | 0900 | 5-28 | 2200 | 8 | 6-04 | 1000 | + | |
| 511 | 5-09 | 5-28 | 1400 | 11 | 5-29 | 0900 | 5-29 | 1500 | 8 | 6-05 | 1100 | + | |
| 512 | ? | 5-27 | 2200 | 12 | 5-28 | 0900 | 5-28 | 2200 | 8 | 6-04 | 1200 | + | |
| 513 | ? | 5-27 | 2200 | 05 | 5-28 | 0900 | 5-28 | 2200 | 8 | 6-04 | 1200 | + | |
| 520 | 5-10 | 5-29 | 0800 | 11 | 5-29 | 1900 | 5-30 | 0800 | 8 | 6-05 | 1700 | + | |

# This chart refers to the year, 1973.

(continued on page 29)

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition.

@ At that hour

Table 4. Implantation sites observed following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Laparotomy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | Implanted | Not implanted |
| 524 | 5-10 | 5-29 | @0800 | 13 | 5-29 | 2000 | 5-30 | 0800 | 8 | 6-05 | 1600 | + | |
| 544 | ? | 5-31 | 1000 | 12 | 5-31 | 1800 | 6-01 | 0900 | 8 | 6-08 | 1100 | + | |
| 574 | 5-18 | 6-06 | 0700 | 12 | 6-06 | 2200 | 6-07 | 0700 | 8 | 6-13 | 1400 | + | |
| 582 | 5-19 | 6-07 | @0700 | 06 | 6-07 | 1900 | 6-08 | 1000 | 8 | 6-15 | 1100 | + | |
| 585 | 5-19 | 6-07 | @0700 | 12 | 6-07 | 1900 | 6-08 | 1000 | 8 | 6-15 | 1100 | + | |
| 588 | 5-19 | 6-07 | 0700 | 11 | 6-07 | 1900 | 6-08 | 1000 | 8 | 6-15 | 1100 | + | |

# This chart refers to the year, 1973.

(continued on page 30)

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition.

@ At that hour

Table 4. Implantation sites observed following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Laparotomy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | Implanted | Not implanted |
| 599 | 5-21 | 6-09 | @0800 | 12 | 6-10 | 1000 | 6-10 | 1000 | 8 | 6-17 | 1200 | + | |
| 600 | 5-21 | 6-09 | @0800 | 10 | 6-10 | 1000 | 6-10 | 1000 | 8 | 6-17 | 1200 | + | |
| 601 | 5-21 | 6-09 | 0800 | 08 | 6-10 | 1000 | 6-10 | 1000 | 8 | 6-17 | 1200 | + | |
| 607 | ? | 6-09 | 0800 | 14 | 6-09 | 1800 | 6-10 | 1000 | 8 | 6-17 | 1200 | + | |

# This chart refers to the year, 1973.

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition.

@ At that hour.

Table 5. Mice in which implantation sites were not observed following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Laparotomy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | Implanted | Not implanted |
| 505 | 5-08 | 5-27 | 0900 | 06 | 5-27 | 2200 | 5-28 | 0900 | 8 | 6-04 | 1000 | | + |
| 507 | 5-09 | 5-28 | @1400 | 12 | 5-29 | 0900 | 5-29 | 1500 | 8 | 6-05 | 1100 | | + |
| 509 | 5-09 | 5-28 | 0900 | 16 | 5-28 | 2000 | 5-29 | 0900 | 8 | 6-04 | 1100 | | + |
| 519 | 5-09 | 5-28 | @1800 | 13 | 5-29 | 0900 | 5-29 | 1800 | 8 | 6-05 | 1100 | | + |
| 523 | 5-10 | 5-29 | 0800 | 09 | 5-29 | 1900 | 5-30 | 0800 | 8 | 6-05 | 1600 | | + |
| 525 | 5-10 | 5-29 | 0800 | 12 | 5-29 | 1900 | 5-30 | 0800 | 8 | 6-05 | 1600 | | + |

# This chart refers to the year, 1973.

(continued on page 32)

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition.

@ At that hour.

Table 5. Mice in which implantation sites were not observed following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Laparotomy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | Implanted | Not implanted |
| 528 | 5-10 | 5-29 | @0800 | 08 | 5-29 | 2000 | 5-30 | 0800 | 8 | 6-05 | 1600 | | + |
| 529 | 5-10 | 5-29 | 0800 | 12 | 5-29 | 1900 | 5-30 | 0800 | 8 | 6-05 | 1600 | | + |
| 532 | ? | 5-29 | 1400 | 09 | 5-29 | 2000 | 5-30 | 0900 | 8 | 6-05 | 1600 | | + |
| 537 | 5-12 | 5-31 | @1200 | 12 | 6-01 | 0900 | 6-01 | 1800 | 8 | 6-08 | 1100 | | + |
| 538 | 5-12 | 5-31 | @1000 | 11 | 5-31 | 2300 | 6-01 | 1000 | 8 | 6-08 | 1100 | | + |
| 539 | 5-12 | 5-31 | 1000 | 10 | 5-31 | 1800 | 6-01 | 0900 | 8 | 6-08 | 1100 | | + |

# This chart refers to the year, 1973.

(continued on page 33)

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition.

@ At that hour

Table 5. Mice in which implantation sites were not observed following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Laparotomy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | Implanted | Not implanted |
| 540 | 5-12 | 5-31 | @1000 | 08 | 5-31 | 2300 | 6-01 | 1000 | 8 | 6-08 | 1100 | | + |
| 542 | 5-12 | 6-01 | 0800 | 09 | 6-01 | 1800 | 6-02 | 0900 | 3 | 6-08 | 1200 | | + |
| 545 | ? | 5-31 | 1000 | 12 | 5-31 | 1800 | 6-01 | 0900 | 8 | 6-08 | 1100 | | + |
| 547 | 5-13 | 6-01 | 0800 | 11 | 6-01 | 1800 | 6-02 | 0900 | 7 | 6-08 | 1200 | | + |
| 549 | 5-13 | 6-01 | @0800 | 12 | 6-01 | 2200 | 6-02 | 0900 | 7 | 6-08 | 1200 | | + |
| 550 | 5-13 | 6-01 | 0800 | 14 | 6-01 | 1800 | 6-02 | 0900 | 8 | 6-08 | 1200 | | + |

# This chart refers to the year, 1973.

(continued on page 34)

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition.

@ At that hour.

Table 5. Mice in which implantation sites were not observed following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Laparotomy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | Implanted | Not implanted |
| 575 | 5-18 | 6-06 | @0700 | 06 | 6-06 | 2200 | 6-07 | 0700 | 8 | 6-13 | 1400 | | + |
| 576 | 5-18 | 6-06 | 0700 | 13 | 6-06 | 2200 | 6-07 | 0700 | 8 | 6-13 | 1400 | | + |
| 581 | ? | 6-06 | 0700 | 12 | 6-06 | 2200 | 6-07 | 0700 | 8 | 6-13 | 1400 | | + |
| 583 | 5-19 | 6-07 | @0700 | 12 | 6-07 | 1900 | 6-08 | 1000 | 8 | 6-15 | 1100 | | + |
| 584 | 5-19 | 6-07 | 1900 | 09 | 6-08 | 0900 | 6-09 | 1000 | 8 | 6-16 | 1100 | | + |
| 586 | 5-19 | 6-07 | 0700 | 10 | 6-07 | 1900 | 6-08 | 1000 | 8 | 6-15 | 1100 | | + |

# This chart refers to the year, 1973.

(continued on page 35)

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition.

@ At that hour

Table 5. Mice in which implantation sites were not observed following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Laparotomy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | Implanted | Not implanted |
| 587 | 5-19 | 6-07 | 0700 | 07 | 6-07 | 1900 | 6-08 | 1000 | 8 | 6-15 | 1100 | | + |
| 590 | ? | 6-07 | 0700 | 07 | 6-07 | 1900 | 6-08 | 1000 | 8 | 6-15 | 1100 | | + |
| 591 | 5-20 | 6-08 | 0900 | 13 | 6-09 | 1000 | 6-09 | 1000 | 8 | 6-16 | 1100 | | + |
| 592 | 5-20 | 6-08 | 0900 | 09 | 6-09 | 1000 | 6-09 | 1000 | 8 | 6-16 | 1100 | | + |
| 593 | 5-20 | 6-08 | 0900 | 10 | 6-08 | 1800 | 6-09 | 1000 | 8 | 6-16 | 1100 | | + |
| 595 | 5-20 | 6-08 | 0900 | 11 | 6-08 | 1800 | 6-09 | 1000 | 8 | 6-16 | 1100 | | + |

# This chart refers to the year, 1973.

(continued on page 36)

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition.

Table 5. Mice in which implantation sites were not observed following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Laparotomy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | Implanted | Not implanted |
| 597 | ? | 6-08 | 0900 | 08 | 6-08 | 1800 | 6-09 | 1000 | 8 | 6-16 | 1100 | | + |
| 598 | 5-21 | 6-09 | @0900 | 12 | 6-10 | 1000 | 6-10 | 1000 | 8 | 6-17 | 1200 | | + |
| 602 | 5-21 | 6-09 | @1200 | 08 | 6-10 | 1000 | 6-10 | 1200 | 8 | 6-17 | 1200 | | + |
| 603 | 5-21 | 6-09 | @1500 | 05 | 6-10 | 1000 | 6-10 | 1500 | 5 | 6-17 | 1200 | | + |
| 604 | 5-21 | 6-09 | 0800 | 12 | 6-09 | 1800 | 6-10 | 1000 | 8 | 6-17 | 1200 | | + |
| 605 | ? | 6-09 | 0800 | 12 | 6-09 | 1800 | 6-10 | 1000 | 8 | 6-17 | 1200 | | + |

# This chart refers to the year, 1973.

(continued on page 37)

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition. (except 603).

@ At that hour

Table 5. Mice in which implantation sites were not observed following post partum mating

| Female's cage | Day-1 of first pregnancy* | Parturition | | | Copulation | | Weaned | | | Laparotomy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Month-day# | Hours | Number in litter** | Month-day# | Hours | Month-day# | Hours | Number | Month-day# | Hours | Implanted | Not implanted |
| 606 | ? | 6-09 | 0800 | 13 | 6-10 | 1000 | 6-10 | 1000 | 8 | 6-17 | 1200 | | + |
| 607 | 5-22 | 6-10 | 0900 | 13 | 6-10 | 1800 | 6-11 | 0900 | 8 | 6-18 | 1000 | | + |
| 610 | 5-22 | 6-10 | 0900 | 11 | 6-10 | 1800 | 6-11 | 0900 | 8 | 6-18 | 1000 | | + |

# This chart refers to the year, 1973.

* Morning vaginal plug was observed.

** Litter size was adjusted to eight per female at parturition.

The test of hypothesis was calculated by the following method.

$H_O$: $P_i = P_e$

$H_A$: $P_i < P_e$

$$Z = \frac{f_i - f_e}{\sqrt{f(1 - f)(1/n_i + 1/n_e)}},$$

where $f = \frac{X_e + X_i}{n_e + n_i}$ and Z is a standard normal observation.

$$Z = \frac{16/55 - 12/30}{\sqrt{(28/85)(57/85)(1/55 + 1/30)}}$$

$$Z = \frac{-0.10909}{0.10668}$$

$$Z = -1.02$$

Based on these data, the observed Z offers no evidence that $P_i < P_e$.

## Discussion

In the initial stages of this study, it was proposed that the delayed implanting female mouse might prove to be a very compatible recepient for ovum transfer experiments. None of the ova transferred to the uteri of the pseudopregnant lactating females on day-5 of lactation pregnancy implanted. The results of this study indicate that such manipulations would not be feasible to expidite that particular technique. This conclusion was reached due to the actual reduced incidence of lactation pregnancy.

The litter size was maintained at eight per lactating female mouse. It was assumed that maximum mammary activity occurs with eight suckling young, and this number can be adjusted easily.

The gestation prolonged 14 days (Table 1) confirms that implantation occurs at the end of the thirteenth day of lactation pregnancy (23) but rejects that implantation does not occur before the sixteenth day of pregnancy (42). These 14 and 11 days of prolonged gestation were longer than the average delay of nine days.

Since a rarity of lactation pregnancies resulted during these initial trials, a decreased

incidence of implantation, conception, and/or ovulation was hypothesized.

The deletion of ineffectual methods was the most important result of the initial procedure. Other workers used lactating foster females or placed the male into the individual female's cage. Both of these methods proved unsatisfactory for the litters were destroyed by the foster mother, true mother, or male . The best method to achieve post partum mating is to place the lactating female with the male any time 12-24 hours post partum.

The incidence of post partum ovulation was not analyzed. This could be accomplished by flushing the oviducts approximately 24 hours post partum in a non-copulated lactating female and observing unfertilized ova.

The incidence of conception is related to sperm motility, fertilization of the oocyte, and the frequency of post partum ovulation. Sperm motility may be influenced by the sloughing of the uterine endometrial mucosa preventing the sperm from reaching the oviduct where penetration of the oocyte

(fertilization) occurs. Male fertility was tested and affirmed during the first pregnancy of the females.

The rate of conception was 0.40 and the upper and lower limits of conception were 0.58 and 0.22, respectively, given a 95% confidence interval. This means that 95 out of 100 times the incidence of conception will be between 0.22 and 0.58.

Implantation sites can be observed on day-5 of lactation pregnancy when the litter is sacrificed 24 hours post partum. However, laparotomy was not performed until after day-5 so that the implantation sites could be more easily recognized.

The rate of implantation was 0.29, and the upper and lower limits of implantation were 0.41 and 0.17, respectively, given a 95% confidence interval. This means that 95 out of 100 times the incidence of implantation would be expected to be between 0.17 and 0.41.

The observed Z, a standard normal observation, in the test of hypothesis offered no evidence that the true percentage of implantation (29%) was less than the true percentage of conception (40%).

## Summary

Copulation was not observed the first 12 hours post partum; but, mating may be observed any time during the next 12 hours that the female is placed with the male. The time of copulation can be witnessed and recorded 12-24 hours post partum.

Twelve mice in a sample of thirty (Tables 2 and 3) conceived during the post partum mating. This was determined by flushing the oviducts and observing ova. The upper and lower limits of conception were 0.58 and 0.22, respectively, given a 95% confidence interval.

Sixteen mice in a sample of fifty-five (Tables 4 and 5) had obserable uterine implantation sites upon laparotomy after day-5 of lactation pregnancy. The upper and lower limits of implantation were 0.41 and 0.17, respectively, given a 95% confidence interval.

Given a standard normal observation, Z, the test of hypothesis offers no evidence that the true percentage of implantation is less than the true percentage of conception.

The post partum lactating mouse does not seem to be a feasible method to expidite ovum transfers nor efficiently increase the mouse population based upon the low incidence of implantation.

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